III. REMARKS

Claim Status

Claims 1, 6-10, 12-13 and 18-39 are under current examination. Claims 1, 6-13 and 18-34 stand rejected. Claims 1, 12, 18 and 31-33 have been amended. Claim 11 has been cancelled. Claims 35-39 are new.

Claim Rejections – 35 USC § 103

Claims 1-2, and 10-13 and claims 1-2, 6-13 and 18-34 remain rejected under 35 U.S.C. 103(a) as being obvious over Margolskee (US-5,817,759, issued 6 October 1998) in view of Yao et al. (US-7,041,457, issued 9 May 2006)' and further in view of Ruiz-Avila et al. (PNAS. July 17 2001. vol.98; No.15: 8868-8873).

a) Applicant's Claims

Applicant discloses and claims a $G_{\alpha q\text{-Gustducin}}$ chimeric G-protein wherein the last 44 amino acids of the $G_{\alpha q}$ protein sequence are replaced with a 44 amino acid unit of Gustducin, G15/gust 44 and G16/gust44. these chimera provide increased signal strength.

The examiner remarked that the requirement for increased signal strength was not contained in the claims at issue. Applicant has now amended the claims to indicate that the claimed compositions up-regulate signal strength, a property not exhibited by the prior art compositions or recognized by the prior art, thus making the claimed compositions substantially

more useful for their intended purpose and providing a surprising advantage over the existing art.

Basis for this amendment appears at paragraphs [0036], [0044]-[0049] of applicant's published application.

b) Summary of Argument

Applicant respectfully traverses the Examiner's rejection of the indicated claims in view of the Margolskee, Yao and Ruiz-Avila references.

Margolskee teaches that Gustducin a subunit variants may comprise polypeptide analogs wherein one or more of the specified amino acids is deleted or replaced or wherein one or more nonspecified amino acids are added.

Up to this point Margolskee is offering no specifics.

Margolskee also teach that the transducins comprise a subfamily of closely related proteins and that the carboxy terminal 60 amino acids of all three proteins gustducin and rod and cone transducins are highly conserved, while the carboxyl terminal 38 amino acids are identical and that the carboxyl terminal identity is of particular importance because it encompasses the site that has been implicated in G protein/receptor interactions.

In addition, Margolskee teach SEQ ID NO:3 which consists of the last 40 amino acids of Gustducin α subunit and is 100%

identical to the last 40 of SEQ ID NO:2 of the instant application.

The criticality or not of the last 44 amino acids of the G protein is at best an invitation to experiment with the 44 amino acid long chain at the end of the much larger g protein. An invitation to modify one or more of 44 amino acids is an invitation to test one or more of the literally millions, if not billions, of combinations and permutations possible when dealing with a chain of 44 individual amino acids, each of which can be individually modified. The image of searching for a needle in a haystack is immediately brought to mine.

Assuming, *arguendo*, that an intrepid experimenter would begin this apparently unrewarding task, where would he start.

The intrepid experimenter may start out, as did the examiner, with Margolskee who teaches the α subunit of a novel taste receptor cell specific G protein, gustducin, or fragments and variants of the a subunit and subtypes of G-proteins, Ga15 and Ga16.

So, the examiner argues, a hypothetical experimenter is going to focus on the α subunit and the last the 40 amino acids at the carboxy end. But Margolskee do not teach chimeric G-proteins comprising the carboxy 40 amino acids of Gustducin α subunit.

Following the examiner's journey, and with knowledge of Ruiz-Avila et al. who teach several biochemical studies suggesting that the interaction of gustducin with its cognate taste receptors is similar to that of transducin with rhodopsin

and that a key result of these studies is that the C terminus of a-gustducin is a critical determinant for its interaction with taste receptors, the next step our experimenter takes is to consider Yao et al.

Yao et al. teach chimeric Gq variants and the isolated nucleic acids encoding the same.

In one embodiment, the chimeric Gq protein variants comprise C-terminal sequences from transducin or G_{odf}. Yao et al. teach our experimenter that, in a preferred embodiment, at least about five amino acids in the C terminus of the Gq protein are replaced by at least about five amino acids from the C terminus of Ga_{olf} or transducin and that up to 44 amino acids of the C terminus of transducin or G_{odf} may be incorporated.

Yao et al. indicates that the C-terminus of Gα proteins can be modified to promote promiscuity of taste receptors. Yao et al. also describe the shared homologies of Gα subunits. Further, Yao et al. also suggest that the gustducin-coupled bitter receptor can be modified to increase promiscuity with regard to GPCR coupling.

Yao et al. report their results in Table 1. Specifically, Yao et al. show that one specific chimeric G-protein MGq(ΔN-HVD-HA)-t44 resulted in bitter taste receptor functionality as did 2 t5 chimeras MGq(ΔN-HVD-HA)-t5 and MGq (HVD-HA)-t5.

Thirteen (13) other variants show little or no functionality. An experimenter extrapolating form Yao et al.'s results would not be pointed to any specific carboxy end change since the -olf5 version didn't work. He would not be sure of the

ΔN portion since it worked twice, had some functionality once and didn't work twice. The HA portion worked 3 times but didn't work 4 times. The HVD portion would appear to be the best bet.

The pertinent part of Yao et al.'s disclosure is that a tail of t5, or t44 combined with a specific construct would work but a tail of olf5 wouldn't work.

Notably, the present claims are directed to a combination with G_{15}/G_{16}, which from Yao is known to give poor signal strength.

> "Similar problems arise when using $G_{\alpha15/\alpha16}$ to identify ligands of ORs and T2Rs (bitter taste receptors) in that (1) calcium responses to odorants are small and quickly desensitized for ORs in $G_{\alpha15/\alpha16}$ transiently transfected cells (Krautwurst et al., 1998); (2) most T2Rs remain orphan using cell lines stably transfected with Gα15 (Adler et al., 2000; Chandrashekar et al., 2000); and (3) threshold concentration of denatonium measured is at least one order higher than expected for bitter receptors, hT2R4 and mT2R8 expressed in cells stably transfected with Ga15 (Adler et al., 2000; Chandrashekar et al., 2000). These problems suggest that the coupling efficiency between ORs/T2Rs and $G_{\alpha15/\alpha16}$ is weak and may vary within the family of ORs and T2Rs."(Yao, col. 2, lines 37-50)

The conclusion drawn by the examiner is that, oblivious to this disclosure, our experimenter would proceed, without untold numbers of experiments rising to the level of non-obviousness, to substitute the specific 44 amino acid sequence tail disclosed by applicant.

But this would require a leap that is logically blocked by Ueda et al. which discloses to our experimenter that results obtained utilizing transducin do NOT apply to gustducin.

For all the general statements about the equivalence of or interchangeability between transducin and gustducin, the actual experimental results, as reported by Ueda et al., found that transducin 5, 11 and 23 amino acid tails of transducin were not equivalent to, nor functional as, the 5, 11 and 23 amino acid tails of gustducin.

Thus, although substitutions of 5 amino acids or more is functional when the tail is transducin, over 80% of the time, Yao et al.'s chimera's are not functional as applicant's chimera even if the leap were made from transducin to gustducin.

The documentary evidence in the record of this application thus refutes the examiner's *post hoc* argument that the disclosure of a tranducin tail renders obvious a gustducin tail.

So even if our experimenter stumbled upon the idea of substituting a different tail comprising gustducin onto his molecule he would be disappointed because his chimera would not be functional until he reached 37 or preferably applicant's claimed 44 amino acid long chain.

Thus, even after this supposed series of multiple serendipitous discoveries, our experimenter would still not reach his goal of reproducing applicant's claimed invention.

This clearly demonstrates that, based on documentary evidence and not examiner or attorney argument, the combination of Margolskee, Ruiz-Aveda and Yao et al. do not sufficiently

provide guidelines to an experimenter skilled in the art to render obvious applicant's claims and that a *prima facie* case of obviousness has not been established.

Tail Length

The disclosure by Yao et al. of a range of substitutions of from 5 to 44 overlaps applicant's substitution of 44 amino acids.

In the case where the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a *prima facie* case of obviousness exists. *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976); *In re Woodruff*, 919 F.2d 1575, 16 USPQ2d 1934 (Fed. Cir. 1990) Further "where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)

But importantly, a particular parameter must first be recognized as a result-effective variable, i.e., a variable which achieves a recognized result, before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation. *In re Antonie*, 559 F.2d 618, 195 USPQ 6 (CCPA 1977)

Applicants can rebut a *prima facie* case of obviousness based on overlapping ranges by showing the criticality of the claimed range. "The law is replete with cases in which the difference between the claimed invention and the prior art is some range or other variable within the claims… . In such a situation, the applicant must show that the particular range is

critical, generally by showing that the claimed range achieves unexpected results relative to the prior art range." *In re Woodruff*, 919 F.2d 1575, 16 USPQ2d 1934 (Fed. Cir. 1990).

Applicant has clearly done this by provision of the Ueda reference and by the disclosure in his own specification, as set forth above. The range set forth in Yao et al. incorporates numerous inoperable species and does not show criticality in the size of the tail.

Teaches away

A *prima facie* case of obviousness may also be rebutted by showing that the art, in any material respect, teaches away from the claimed invention. *In re Geisler*, 116 F.3d 1465, 1471, 43 USPQ2d 1362, 1366 (Fed. Cir. 1997).

Here Yao is teaching that the <u>preferred</u> substitution size is 5 amino acids, about one-tenth the number claimed by applicant.

Inherency

Furthermore, the fact that a certain result or characteristic <u>may</u> occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art).

In re Oelrich, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). "To establish inherency, the extrinsic evidence

'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' "

Metabolite Labs., Inc. v. Lab. Corp. of Am. Holdings, 370 F.3d 1354, 1367, 71 USPQ2d 1081, 1091 (Fed. Cir. 2004) stands for the proposition that "[a] prior art reference that discloses a genus still does not inherently disclose all species within that broad category" but must be examined to see if a disclosure of the claimed species has been made or whether the prior art reference merely invites further experimentation to find the species.

Applicant previously stressed that no predictions are possible regarding whether specific chimeric embodiments are functional, [i.e. whether they would be promiscuous and transmit a signal to the receptor strong enough to be useful in a screening method for the embodiments claimed and for even the more similar ones not currently claimed.

To work in a screening method, binding and/or signal transduction activity are both necessary, but not sufficient, prerequisites.

Changes in the constituents of the chimera may affect the three dimensional shape and other required functionalities, including, in particular, promiscuity, which the present invention successfully increases. An increase in promiscuity of a given chimeric protein is at least as unpredictable as its

general functionality. The same principle also applies to signal strength.

The examiner argues that Ueda et al. does not stand for the proposition originally advanced by applicant – that Ueda et al. fully contradicts the examiner's position in that <u>some</u> of Ueda's constructs do work.

By the same token however, some of Ueda's constructs do not work. The disclosure of Yao teaches chimeric Gq variants where, in a preferred embodiment, from 5 to 44 amino acids in the C terminus are substituted. Yao thus discloses a genus [range] where over 80% of the species in his genus are inoperable.

The Examiner's argument and basis for rejection of applicant's claims requires predictability. To succeed is establishing a *prima facie* case of obviousness, all of these variants, which have a transducin C-terminus of 5 and up to 44 amino acids as taught by Yao, i.e. including the -t5 variant, would have to actually work giving robust signal strength in a screening method.

<u>They did not.</u>

On the contrary, Ueda reports that none of these variants responded to different T2R receptors with known ligands; contrary to the position taken by the examiner, neither of the chimeric proteins having shorter C-termini worked, thereby clearly demonstrating a high level of unpredictability.

The experimenter attempting to develop a functional chimera, efore trying it out experimentally, i.e. without

applying hindsight, would find it impossible to predict which domains will continue to bind, interact and transmit their signal with all interacting partners in their new three dimensional environment. A chimeric protein might lose or acquire new unwanted functionality leading to incompatibility with any one of its partners, either in binding, in interaction or in signal transmission.

For example, G-proteins are heterotrimeric and consist of alpha, beta and gamma subunits, so any change would be required not to significantly affect the interaction with the other subunits. Yao et al. is in agreement with this point, stressing the central part of this type of G-protein with various components in signaling:

> "Intracellular signaling is mediated through various effector enzymes, including cGMP phosphodiesterase, phospholipase C, adenylate cyclase, etc. (see Kinnamon & Margolskee, 1996, Curr. Opinion Neurobiol. 6: 506 513). Most effector proteins interact with the Gα, although Gβ γ subunits also contribute to the specificity of receptor-G protein coupling (Xu et al., 1998, J. Biol. Chem. 273(42): 27275 79)." (Yao, col. 1, lines 35-42)

Further, even if general functionality remained, it is even more unclear whether the chimeric protein would have the necessary signal strength for a screening method or the desired increased promiscuity, e.g. being activated and transmitting the signal of both bitter and sweet receptors.

Ueda et al. did not set out to establish a system of increased promiscuity and signal strength. Instead Ueda was

researching the relative importance of the various domains, and appears rather surprised by the findings, especially by the fact that the G16/gust23 variant did not work, even though the a5 helix was believed to be the major factor:

> "In contrast, G16/gust23 that contained the a5 helix of gustducin appeared not to associate, although numerous studies have attested to the importance of the α5 helix in receptor coupling. Similarly, G16/gust11 and G16/gust5 did not cause T2R activity. These results indicated that the a5 helix and extreme C terminus of gustducin were insufficient for detection of T2R activities, and the β6 sheet, in addition to the α5 and C-terminal β-sheet, is indispensable for signal transduction of T2Rs." (Ueda 7379, top of right col.)

This indispensability was not previously known, Ueda being published after the priority date of the present application.

In view of Yao, Ueda's results may at first glance seem somewhat surprising, <u>provided one assumes a predictability that the field simply does not have</u>. The skilled person might hope and try out variations to see which one works, but would certainly not do so with a reasonable expectation of success. Notably, Yao exemplified and specifically disclosed only the mouse Gαq-t5 and mouse Gαq-t44 variants (but not hGαq-t5 nor hGαq-t44, nor any mouse or human G16 variant, compare table I in Yao).

Further, Yao tested only the mT2R5 receptor, thereby failing to show increased promiscuity.

The examiner asserts that "regar[d]less of the overall "low 58% homology" between gustducin and transducin, the portion important for function (44 aminoacids of the carboxy terminus), there is a much greater homology."

However, the C-terminus, or any component, cannot be considered in isolation, and a high homology is not necessarily an indicator of predictability and likelihood of success.

The irrelevance of a high degree of homology and the high degree of unpredictability in the art is further demonstrated by one of the prior art documents cited by the Examiner, Ruiz-Avila, which is discussed in Ueda. A gustducin mutant that is <u>identical but for the exchange of only one amino acid</u> in the extreme C-terminus results in a loss of the ability to activate receptors, as shown in the discussion of Ruiz-Avila in Ueda, Ueda page 7380:

> "Indeed, a gustducin mutant containing a glycine-to-proline substitution at position —3 can bind to taste receptor Gβγ subunits and the effector, but it cannot be activated by receptors (Ruiz-Avila et al., 2001). Therefore, the extreme C terminus may also play an important role in transduction via the gustducin, Gαt1, Gαt2, and Gαi2 of T2R taste receptors." (Ueda, left col., lines 11-16)

This highlights the significance of the difference between transducin and gustducin (6 aminoacids) in the short C-terminal stretch of only 44 aminoacids in the claimed chimeric proteins.

These are combined with a different backbone, G16/G15, while Yao merely disclosed chimeric proteins comprising the two

mouse variants of a different specific Gαq class protein, the one that gave the class its name, Gαq: MGq(DeltaN-HVD-HA)-t5 and MGq(DeltaN-HVD-HA)-t44 (both using transducin C-terminal sequences, with the five "t5" amino acids of transducin being identical to those of gustducin).

Applying the Examiner's modular view and dismissing potential interactions of C-terminal part with backbone, -t5 chimeric proteins (MGq(DeltaN-HVD-HA)-t5 and G16/gust5) being more similar to each other than the ones currently claimed, as at least the C-terminal part is identical, the difference being "only" in the combination with a different backbone, G16-t5 aka G16-gust5 would be predicted to work.

However, even if using the Gα16-t5 (Gα16/gust5) chimeric variant identical in the "critical" C-terminal part, the results differ significantly, as Ueda shows for the Gα16-gust5 chimeric variant in comparison to the Gα16-t44 variant.

This further demonstrates that not even partial identity (t5/gust5) of a "critical" part is a good indicator of transferability, and success cannot be predicted.

Accordingly, the differing C-terminus of the presently claimed proteins (Yao's t44 C-terminus differs from the C-terminus of the claimed gust44 chimera in 6 of its 44 aminoacids, having a homology of 86.3% in the C-terminal part) <u>is even less predictable</u> in its interaction with the backbone, especially with the different backbone of G16/G15.

Ueda's results demonstrate that <u>homology cannot be considered in "modules"</u>, and accordingly the total homology

(which is very low, 57%, as previously explained) may be relevant as well and has to be considered in addition to the partial homology. Receptor proteins function in the three-dimensional context with their G-protein interaction partners.

When cutting out a specific "module" and transplanting it elsewhere, regardless of a higher degree of homology (or even identity, as shown by Ueda for the G16-t5/-gust5) in the transplant, this does not mean it will work in its new surroundings, e.g. with a new G16/G15 backbone. In particular, it is not predictable whether the new variant would provide a signal strength useful for screening or increase promiscuity.

G16 or its ortholog G15, both share a low degree of homology of less than 57% similarity to Gαq, which, according to Yao, is a high divergence that should result in significant differences in efficiency and selectivity of receptor coupling:

> "Protein sequence similarity between Gαq and G α15/Gαq16 is less than 57% (FIG. 1). Accordingly, such high divergence should result in significant differences in efficiency and selectivity of receptor coupling. The identification of functionally active Gq protein variants could allow for the pharmacological and genetic modulation of sensory transduction pathways." (Yao, col. 4, lines 21-27)

Notably, Yao mentions G15/G16 only in general, enumerating proteins belonging to the same class as Gαq, and in particular points out the differences to Gαq ("Protein sequence similarity ... less than 57%", "high divergence"). Even for the variants disclosed (Gαq itself), no increased promiscuity is shown, as the variants are tested with only one receptor (compare Yao's table 1).

Furthermore, Yao points out problems with Gαq class proteins, and especially G16/G15, which are not true universal adaptors. In particular, signal strength is a problem:

> "Despite their promiscuity, however, Gαq class subunits do not mediate all GPCR--effector interactions. For instance, human Gα16 and its murine counterpart Gα5 are promiscuous G proteins in that they couple to GPCRs of different G protein families (Offermanns and Simon, 1995; Negulescu et al., 1997). However, they are not true universal adapters for GPCRs in that there are at least 11 GPCRs reported to be incapable of activating G.alpha.15/G.alpha.16 (Wu et al., 1992; Arai et al., 1996; Kuang et al., 1996; Lee et al., 1998; Parmentier et al., 1998; Mody et al., 2000). Similar problems arise when using Gα15/α16 to identify ligands of ORs and T2Rs (bitter taste receptors) in that (1) calcium responses to odorants are small and quickly desensitized for ORs in Gα15/α16 transiently transfected cells (Krautwurst et al., 1998); (2) most T2Rs remain orphan using cell lines stably transfected with Gα15 (Adler et al., 2000; Chandrashekar et al., 2000); and (3) threshold concentration of denatonium measured is at least one order higher than expected for bitter receptors, hT2R4 and mT2R8 expressed in cells stably transfected with Gα15 (Adler et al., 2000; Chandrashekar et al., 2000). These problems suggest that the coupling efficiency between ORs/T2Rs and Gα15/α16 is weak and may vary within the family of ORs and T2Rs." (Yao, col. 2, lines 28-50)

Furthermore the paralogs (G16/15 versus Gαq) are not very conserved, suggesting distinct functions according to Yao, even though some activities are similar:

> "Signaling specificity among α subunits of the same class having similar biochemical functions is not well understood in vivo. For instance, the Gαq (Gq) class includes four proteins expressed in mammals, called Gαq, Gα11, Gαq14, and Gαq15 (in mice, Gα16 in humans). Whereas orthologs of these subunits are highly

> conserved across species (99, 97, 96 and 85% identity, respectively), paralogs of these subunits (expressed in the same species) are not as conserved. This suggests that each type of subunit in the Gq class has a distinct function, however, when transfected into Sf9 cells, the subunits stimulated phospholipase C with similar potency and showed similar activities (Nakamura et al., 1995, J. Biol. Chem. 270: 6246 6253). Xu and colleagues subsequently showed by gene knockouts in mice that Gq.sub.60 subunits promiscuously couple to several different receptors in various cell types (1998, J. Biol. Chem. 273(42): 27275)." (col. 1, line 56 to col. 2, line 4)

This would appear to discourage the skilled artisan from replacing Gαq with G16/G15, or at least indicate unpredictability expecting different results, in particular if the C-terminal module is replaced as well.

In any case, it remains that the result is unpredictable, as shown by Ueda's G16gust5 variant which does not appear to work, and certainly works differently from G16gust44 which Ueda tested in parallel.

Claim Rejection – 35 USC 112, 1st and 2nd paragraph

This is essentially a nomenclature issue. The examiner queries the adoption of a different naming convention in applicant's last response. Applicant has slightly modified the designation and adopted the revised naming convention based on articles appearing in the literature which utilize the convention adopted by applicant. Regardless of the convention utilized applicant states that the molecule named utilizing the revised convention is identical to the molecule named in accordance with the original name.

Based on the evidence submitted with this response, applicant has retained the modified revised nomenclature but, in the interests of maximizing clarity, will revert to the original naming convention if the examiner, after reviewing the articles submitted herewith, determines that the public would be best served by reversion to the original naming convention used by applicant.

Conclusion

Since the existence of a *prima facie* case of obviousness therefore is contingent upon the correctness of the examiner's position 1) as to whether or not the references cited by the examiner show a high enough degree of predictability to render applicants instant claims obvious and 2) secondarily, whether the degree of homology described in the art is at a level sufficient to allow one skilled in the art to produce applicant's claimed compositions with the expectation that the claimed compositions would function as successfully as they do for their intended purposes and applicant has demonstrated by extrinsic factual scientific evidence that neither predictability nor homology exist, the *prima facie* case upon which the rejection is based has been negated and

Accordingly, favorable reconsideration of and withdrawal of the current rejection of the present claims is solicited.

Should the Examiner in charge of this application believe that telephonic communication with the undersigned would meaningfully advance the prosecution of this application towards

allowance, the Examiner is invited to contact the undersigned at his earliest convenience.

Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

Respectfully submitted,

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